58, lines 27-33. Accordingly, the amendments do not introduce new matter and entry thereof is respectfully requested.

Applicant has set forth above the amended claims in clean form as required under 37 C.F.R. § 1.121(c)(i). Applicant also attach the appendix "Marked Up Claim Amendments" with the claim amendments indicated with brackets and underlining as required under 37 C.F.R. § 1.121(c)(ii).

Regarding the disclosure in the parent provisional application 60/083,154, filed April 27, 1998, Applicant respectfully disagrees with the assertion in the Office Action on page 2 that claims 3, 4, 20, 21 and 29-32 are not supported by the parent provisional application. In particular, the parent provisional application, which included Gerloni et al., DNA Cell Biol. 5:611-625 (1997), is enabling for these claims. Gerloni et al. (DNA Cell Biol., 1997) teach that administering a nucleic acid molecule having an expression element operationally linked to a nucleic acid sequence encoding a heterologous epitope to lymphoid tissue stimulated an immune response in mice (page 615, column 2, first paragraph, and Table 1, page 614). Specifically, as shown in Table 1, all of the mice of group I, which were inoculated in lymphoid tissue with a nucleic acid encoding a heterologous epitope of the γ1WT protein, showed an antibody response as measured in sera collected 21 days after inoculation. Furthermore, a single intrasplenic inoculation of a nucleic acid encoding the γ1WT protein induced an IgM primary immune response (page 618, column 2, last paragraph, and Figure 8a, page 621).

When these mice were boosted with the γ1WT protein (secondary immune response), a strong IgG_1 and IgG_{2b} response was mounted.

Regarding the alleged lack of teachings pertaining to *in vivo* methods, the parent provisional application teaches that the claimed methods resulted in a long lasting humoral immunity against the transgene product and induction of immunologic memory (Gerloni et al. DNA Cell Biol., 1997, page 621, column 2). Gerloni et al.(DNA Cell Biol., 1997) further teaches that the claimed methods can be used to treat a condition, in particular indicating that tissue specific targeting of spleen lymphocytes is an effective way to initiate a humoral immune response (page 622, column 1, first partial paragraph). One skilled in the art would have recognized that an initiation of a humoral immune response is an effective method for treating a variety of conditions. Therefore, the parent provisional application teaches *in vivo* methods. Accordingly, Applicant respectfully submits that provisional application 60/083,154 provides an enabling disclosure for the claims.

The rejection of claims 3, 4, 18-21 and 29-32 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement is respectfully traversed. Applicant respectfully submits that the specification provides sufficient description and guidance to enable the claimed methods and compositions. The specification teaches methods for stimulating an immune response for treating a variety of medical conditions. For example, the specification teaches that the methods of the invention can be used to stimulate an immune response to pathogens, tumors and

pathological processes, including a variety of specifically recited diseases and conditions (page 25, line 8, to page 26, line 22).

Applicant respectfully disagrees with the assertion in the Office Action that teachings in the specification for using the claimed invention in mice cannot be extrapolated to any and all species. Applicant submits that one skilled in the art would have recognized that the guidance provided in the specification is relevant to any and all species having lymphoid tissues. The specification teaches that the invention can be used for human and other animals where use of the invention for the production of human and animal vaccines is described (page 26, lines 17-20). Moreover, one skilled in the art would have recognized that the teaching provided for use of the claimed invention in mice can be extrapolated to any and all species to include humans because the invention overcomes any perceived unpredictability in prior methods for using DNA vaccines. Specifically, the claimed methods are directed to administering a nucleic acid molecule encoding one or more heterologous epitopes to a lymphoid tissue. The specification teaches methods of direct delivery to lymphoid tissues including for example injection into lymphoid tissues (page 68, line 10, through page 69, line 4) and *ex vivo* gene transfer to lymphoid cells (page 58, line 12, to page 59, line 9). Thus, the methods are directed to initiating immunity at the site of immune induction.

In contrast to the methods taught in the specification, prior methods of DNA delivery such as those described by

McCluskie et al., Mol. Med. 9:267-300 (1999), relied upon indirect delivery methods and ubiquitous expression from a variety of cell types. McCluskie et al. describes DNA administration by eight injection routes including intraperitoneal, intradermal, intraveneous, intramuscular, intraperenial, subcutaneaous, sublingual, and vaginal wall; and six non-injected sites including intranasal inhalation, intranasal instillation, intrarectal, intravaginal, ocular and oral feeding. McCluskie et al. concludes that delivery of DNA by these indirect means in mice was not always predictive of results for non-human primates. However, McCluskie et al. does not describe any method of direct delivery much less direct delivery to lymphoid tissue, nor does McCluskie disclose or suggest that direct methods of delivery would be unpredictable.

Applicant respectfully disagrees with the assertion in the Office Action that the methods taught in the specification require boosting with γ_1NANP/GM-CSF. The specification teaches that primary immunization with the nucleic acid molecules of the invention leads to an immune response. For example, the specification teaches that primary immunization led to production of both IgG1 and IgM (pages 105-107). Regarding Figure 20, note that Panel A shows total antibody titer and Panel B shows IgG1. Therefore, in this experiment, the GM-CSF construct affected the specific subtype of IgG1. However, the top panel clearly shows that an immune response was observed with DNA alone. Furthermore, as shown in Figure 24, IgM was observed with DNA alone in the absence of boosting. With boosting, DNA alone also increased the IgG1 titer. Therefore, the specification provides

sufficient description and guidance to enable the claimed methods for stimulating an immune response.

Applicant respectfully submits that the specification provides sufficient description and guidance to enable the use of hematopoietic cell expression elements in a variety of lymphoid cells. The specification teaches on page 28, lines 22-30, that hematopoietic cell expression elements allow expression in a cell of hematopoietic origin including for example, a B cell, T cell or dendritic cell. According to the teaching in the specification, one skilled in the art would have been able to make and use a variety of hematopoietic cell expression elements in the claimed invention to express one or more heterologous epitopes. Specifically, the nucleic acid sequences for hematopoietic cell expression elements are known in the art and can be operationally linked to a nucleic acid encoding one or more heterologous epitopes using routine methods such as those taught in the specification. Such routine methods are taught in the specification including, for example, on page 63, line 11, through page 64, line 10, where construction of four plasmid vectors having hematopoietic cell expression elements operationally linked to nucleic acid sequences encoding one or more heterologous epitopes is taught. As described on page 68, line 14, through page 69, line 21, and shown in Table 1, intrasplenic innoculation of a V_H region-γ1C region chimaera under the control of a hematopoietic cell expression element resulted in an immune response in 4 of 4 mice and a significant antibody titer. According to the guidance provided in the specification, one skilled in the art would have recognized that

methods taught for making and using hematopoietic cell expression elements in B cells can be used for expression in other lymphoid cells including, for example, T cells and dendritic cells.

Nevertheless, to further prosecution, Applicant has amended claims 3 and 18 to recite that the expression element is a B cell expression element. As acknowledged in the Office Action on page 5, last paragraph, a B cell expression element is enabled.

Applicant respectfully submits that the invention overcomes the factors allegedly described by Eck et al., Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Chapter 8 (1995) as barriers to previous methods of *in vivo* gene delivery. The factors described by Eck et al. as requiring consideration for DNA delivery are not relevant to the claimed methods. Specifically, Eck describes the factors in regard to indirect methods of DNA delivery. In contrast, the claimed methods are directed to administering a nucleic acid molecule to a lymphoid tissue directly including, for example, by direct injection or *ex vivo* delivery as described above. The invention overcomes factors described by Eck et al. including, for example, distribution of the DNA following administration, the fraction of vector taken up by the target cell population and the rate of degradation of the DNA because DNA is directly administered to a lymphoid cell or tissue thereby reducing the distance required for DNA to travel and opportunities for improper distribution as well as reducing the time for any degradation of DNA prior to cell uptake. Furthermore, factors pertaining to transgene

expression and described by Eck et al. do not pose barriers for the methods of the invention because, as described on page 44, lines 9-15, the fact that the transgene is harbored in B lymphocytes insures a protracted synthesis and secretion of transgenic immunoglobulins in quantities and form sufficient for the initiation of immunity and the establishment of immunologic memory.

Applicant respectfully disagrees with the assertion in the Office Action that the specification fails to provide an enabling disclosure for the use of any route of administration or target tissue other than intrasplenic. The specification teaches that the methods of the invention can be used to deliver a nucleic acid molecule to a variety of secondary lymphoid tissues. For example, the specification teaches on page 26, lines 24-31 that a secondary lymphoid tissue target can include spleen, lymph nodes, mucosa associated lymphoid tissue, nasal associated lymphoid tissue and urogenital lymphoid tissue. Methods of administering a nucleic acid molecule are taught in the specification including, for example, on page 26, line 31, through page 27, line 3, which describes direct injection into lymphoid tissues such as spleen or lymph nodes. Additionally, administration by methods of *ex vivo* gene transfer to lymphoid cells is taught on page 58, line 12, through page 59, line 9.

Moreover, the claimed methods of administering a nucleic acid molecule to a lymphoid tissue can be used to stimulate an immune response as demonstrated in the specification, for example, on page 68, line 28, to page 69, line

1, which teaches that after a single intraspleen inoculation, a marked antibody response was seen. Furthermore, as shown in Table I on page 69 of the specification, direct inoculation in the spleen provided significant increase in both the number of responders and amount of antibody titer compared to inoculation of the same nucleic acid molecule by indirect delivery methods such as intramuscular, intravenous and subcutaneous injection. The specification further teaches that the expression was stable in stating on page 44, lines 16-29, that after a single intraspleen inoculation, the transgene persisted functionally for four months, consistent with the life span of a long-lived B lymphocyte.

Furthermore, any description of unpredictability in methods of DNA delivery by Miller, FASEB J. 9:190-199 (1995), Deonaraian, Expert Opin. Ther. Pat. 8:53-69 (1998), or Verma, Nature 389:239-242 (1997) is not relevant to the methods taught in the specification for administration of a nucleic acid molecule to a lymphoid tissue. Miller, Deonarian and Verma only describe DNA delivery problems as they apply to targeting of the DNA to appropriate cells when indirect methods of DNA administration are employed. However, Miller describes localized delivery of DNA and *ex vivo* gene transfer as successfully overcoming unpredictability in targeting by other indirect methods of gene delivery. Specifically, Miller states in the paragraph bridging pages 197-198 that

> [Thus,] several such trials (for example, for treatment of ADA deficiency, HIV infection,

> or cancer) have used specific cell populations that have been removed from the patient and infected in vitro by non targeted amphotropic retroviruses before being returned in vivo. Further levels of targeting have been achieved in some cases by careful choice of the patients cells; for instance, ex vivo transduction of tumor infiltrating lymphocytes with potentially tumoricidal genes has been proposed as a means of delivering their products to tumor deposits at much higher concentrations than would otherwise be possible.
>
> In contrast to ex vivo manipulation of target cells where the vector requires very little, if any intrinsic targeting capability, there are an increasing number of protocols in which genes are delivered directly to patients in vivo (such as for treatment of cystic fibrosis and cancer).

Thus, Miller describes *ex vivo* gene transfer and direct delivery *in vivo* as overcoming unpredictability in gene targeting by other methods of DNA delivery. Thus, as described above with regard to Eck et al., direct intrasplenic injection of nucleic acid molecules and *ex vivo* gene transfer into lymphoid cells overcome targeting problems associated with indirect gene delivery methods. Any alleged unpredictability described in the cited references is further obviated by targeting to B cells with a nucleic acid containing a B cell expression element.

In conclusion, the specification provides sufficient description and guidance to enable the claimed methods of administering a nucleic acid molecule to any lymphoid tissue and compositions. Accordingly, Applicant respectfully requests that

rejection of claims 3, 4, 18-21 and 29-32 under 35 U.S.C. § 112, first paragraph, be withdrawn.

Rejection of claims 3, 4, 18-21 and 29-32 under 35 U.S.C. § 112, second paragraph, allegedly because the metes and bounds of "hematopoetic expression element" is unclear, is respectfully traversed. Applicant respectfully submits that the term "expression element" is defined on page 28, lines 15-18 as a nucleic acid regulatory element capable of directing expression of genetic element such as an epitope. A "hematopoietic cell expression element" is defined on page 28, lines 22-24 as a promoter or enhancer that functions in hematopoietic cells. Thus, the term includes, but is not limited to, a class of expression elements, any of which could be used with the claimed invention to elicit an immune response or an expression element located within the major intron of an immunoglobulin heavy chain gene. Nevertheless, to further prosecution, Applicant has amended claims 3 and 8 to recite B cell expression element. Accordingly, Applicant respectfully submits that this rejection has been rendered moot and requests that this rejection be withdrawn.

The rejection of claims 3, 4, 18 and 19 under 35 U.S.C. § 102(b) as allegedly anticipated by Gerloni et al., Nat. Biotech., 15:876-881 (1997), is respectfully traversed. The § 102 rejection has been made under § 102(b). However, for the reasons discussed above, claims 3, 4, 18 and 19 are entitled to the priority date of the provisional application. Thus, because Gerloni et al., Nat. Biotech., 15:876-881 (1997) was published

less than a year before the priority date of the subject application, Applicant will respond to the § 102 rejection as if it were made on the basis of § 102(a).

Submitted herewith is a Rule 132 Declaration of Dr. Maurizio Zanetti. The Declaration of Dr. Zanetti declares that the co-authors of the Gerloni et al., Nat. Biotech., 15:876-881 (1997) publication were working under the direction and supervision of Dr. Zanetti. Thus, under *In re Katz*, 215 USPQ 14 (CCPA 1982), the Gerloni et al., Nat. Biotech., 15:876-881 (1997) publication is not available as prior art. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

The rejection of claims 18 and 19 under 35 U.S.C. § 102(b) as allegedly anticipated by Gerloni et al., DNA Cell Biol., 16:611-625 (1997), is respectfully traversed. The § 102 rejection has been made under § 102(b). However, for the reasons discussed above, claims 18 and 19 are entitled to the priority date of the provisional application. Thus, because Gerloni et al., DNA Cell Biol., 16:611-625 (1997) was published less than a year before the priority date of the subject application, Applicant will respond to the § 102 rejection as if it were made on the basis of § 102(a).

Submitted herewith is a Rule 132 Declaration of Dr. Maurizio Zanetti. The Declaration of Dr. Zanetti declares that the co-authors of the Gerloni et al., DNA and Cell Biology, 16:611-625 (1997) publication were working under the direction

and supervision of Dr. Zanetti. Thus, under *In re Katz*, 215 USPQ 14 (CCPA 1982), the Gerloni et al., DNA and Cell Biology, 16:611-625 (1997) publication is not available as prior art. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

The rejection of claims 3, 4, 18-21 and 29-32 under 35 U.S.C. § 102(b) as allegedly anticipated by Gerloni et al., Eur. J. Immunol. 28:516-524 (1998), is respectfully traversed. The § 102 rejection has been made under § 102(b). However, because Gerloni et al., Eur. J. Immunol. 28:516-524 (1998), was published less than a year before the priority date of the subject application, Applicant will respond to the § 102 rejection as if it were made on the basis of § 102(a).

Submitted herewith is a Rule 132 Declaration of Dr. Maurizio Zanetti. The Declaration of Dr. Zanetti declares that the co-authors of the Gerloni et al., Eur. J. Immunol. 28:516-524 (1998) publication were working under the direction and supervision of Dr. Zanetti. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

The rejection of claims 3, 4, 18-21 and 29-32 under 35 U.S.C. § 102(a) as allegedly anticipated by Gerloni et al., Eur. J. Immunol. 28:1832-1838 (1998), is respectfully traversed. Submitted herewith is a Rule 132 Declaration of Dr. Maurizio Zanetti. The Declaration of Dr. Zanetti declares that the co-authors of the Gerloni et al., Eur. J. Immunol. 28:1832-1838 (1998) publication were working under the direction and

supervision of Dr. Zanetti. Thus, under *In re Katz*, 215 USPQ 14 (CCPA 1982), the Gerloni et al., Gerloni et al., Eur. J. Immunol. 28:1832-1838 (1998) publication is not available as prior art. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

The rejection of claims 3, 4, 18 and 19 under 35 U.S.C. § 103(a) as allegedly obvious over U.S. patent Nos. 5,508,386 and 5,583,202 in view of Banerji et al., Cell 33:729-740 (1983), is respectfully traversed. Applicant respectfully submits that the claimed methods and compositions are unobvious over the cited references. In particular, both the '386 and '202 patent describe the use of antibodies expressed *in vitro* and purified over immunoaffinity columns to elicit an immune response. Specifically, the '386 and '202 patents describe that as early as 30 days after the first immunization with an engineered antibody expressing the NANP tetrapeptide within the CDR3 region of the H chain, rabbits immunized with the same engineered antibody produced anti-NANP antibodies, and the titer rose following a booster ('386 patent, column 10, lines 49-66; '202 patent, column 10, line 66, to column 11, line 17). Therefore, there would have been no motivation to use additional expression elements. Furthermore, neither patent teaches or suggests administration to a lymphoid tissue.

Furthermore, Benerji et al. does not cure the deficiencies of the '386 and'202 patents. At best, Banerji et al. appears to describe expression with lymphocyte specific cellular enhancer elements to cultured cells using synthetic

promoters. However, there is no teaching or suggestion of administering to a lymphoid tissue a nucleic acid molecule comprising an expression element operationally linked to a nucleic acid sequence encoding one or more heterologous epitopes, wherein the expression element comprises a B cell expression element.

Regarding claims 18 and 19, Applicant respectfully submits that the references taken alone or in combination do not teach or suggest the nucleic acid molecule claimed in amended claims 18 and 19. Applicant respectfully submits that Banerji et al. does not cure the deficiencies of the '386 and '202 patents. Absent any teaching or suggestion in the references, when taken alone or in combination, the invention would not have been obvious. Accordingly, applicant requests that rejection of claims 3, 4, 18 and 19 under 35 U.S.C. § 103(a) be withdrawn.

CONCLUSION

In light of the amendments and remarks herein, Applicant submit that the claims are now in condition for allowance and respectfully request a notice to this effect. The Examiner is invited to call the undersigned agent or Cathryn Campbell if there are any questions.

Respectfully submitted,

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APPENDIX A

In the claims:

3. (Amended) [The method of claim 1,] A method for stimulating an immune response, comprising administering to a lymphoid tissue a nucleic acid molecule comprising an expression element operationally linked to a nucleic acid sequence encoding one or more heterologous epitopes, wherein said expression element comprises a [hematopoietic] B cell expression element.

4. (Amended) The method of claim 3, wherein said expression element functions in a [cell selected from the group consisting of] B cell[, T cell, and dendritic cell].

18. (Amended) A nucleic acid molecule comprising a [hematopoietic] B cell expression element operationally linked to a nucleic acid sequence encoding a heterologous polypeptide[.] , wherein said B cell expression element comprises a B cell promoter and enhancer.

19. (Amended) The nucleic acid molecule of claim 18, wherein said expression element functions in a [cell selected from the group consisting of] B cell[, T cell, and dendritic cell].